

huntingtin performs this function less well, there is an increase in HIP1-modulated cell death in individuals with an expanded repeat in the HD gene. These observations offers several possibilities for therapy. First, because the function of huntingtin apparently depends at least in part on the ability to interact with HIP-apoptosis modulating proteins, added expression (e.g., via gene therapy) of normal (non- expanded) huntingtin or of the HIP-binding region of huntingtin should provide a therapeutic benefit. Other DED-interacting peptides may also be used to mask and reduce the interaction of HIP-apoptosis modulating proteins with the death signaling complex. Alternatively, a mutant form of HIP-protein from which the DED has been deleted might be introduced, for example using gene therapy techniques. Because HIP-apoptosis modulating proteins have been shown to self-associate, a protein with a deleted DED may compete with endogenous HIP-protein in the formation of these associations, thereby reducing the amount of apoptotically-active HIP-protein. --

At page 5, lines 3-14, please amend paragaraph as follows:

-- After introducing the plasmids into Y190 yeast host cells, transforming the host cells with an adult human brain Matchmaker™ (Clontech) cDNA library coupled with a GAL4 activating domain, and selecting for the expression of two detectable marker genes to identify clones containing genes for interacting proteins, the activating domain plasmids were recovered and analyzed. As a result of this analysis, three different cDNA fragments were identified as encoding for HD-interacting proteins and designated as HIP 1, HIP2 and HIP3. The nucleic acid sequence of HIP I, as originally recovered in the yeast two-hybrid assay, is given in Seq. ID. No 1. The polypeptide which it encodes is given by Seq. ID No. 2. Further investigation of the HIP I cDNA resulted in the characterization of ~~a longer region of cDNA totaling 4795 bases~~ a longer region of cDNA totaling 4796 bases and a corresponding protein, the sequences of which are given by Seq ID Nos. 3 and 4, respectively. A further portion of the HIP I protein was characterized, extending the length to the complete protein sequence of 1090 amino acids (Seq. ID No. 5) The cDNA molecules encoding HIP-apoptosis modulating proteins, particularly those encoding portions of HIP I, can be explored using oligonucleotide probes for example for amplification and sequencing. In addition, oligonucleotide probes complementary to the cDNA can be used as diagnostic probes to localize and quantify the presence of HIP I DNA. --

At page 11, lines 20-24, please amend as follows:

-- The yeast strain Y190 (MATa leu2-3,112, ura3-52, trp1-901, his3-A200, ade2-101, gal4Agal8OA, URA3::GAL-IacZ, LYS2::GAL-HIS3,cyc') was used for all transformations and assays. Yeast transformations were performed using a modified lithium acetate transformation protocol and grown at 30-°C using appropriate synthetic complete (SQ dropout media. --

At page 12, lines 5-18, please amend as follows:

-- cDNAs from an human adult brain MatchmakerTM cDNA library (Clontech) was transformed into the yeast strain Y190 already harboring the 44pGBT9 construct. The transformants were plated onto one hundred 150 min x 15 mm circular culture dishes containing SC media deficient in Trp, Leu and His. The herbicide 3-amino- triazole (3-AT) (25mM) was utilized to limit the number of false His⁺ positives (3 1). The yeast transformants were placed at 30-°C for 5 days and P-galactosidase filter assays were performed on all colonies found after this time, as described above, to identify P- galactosidase⁺ clones. Primary His⁺/P-galactosidase⁺ clones were then orderly patched onto a grid on SC -Trp/-Leu/-His (25 mM 3AT) plates and assayed again for His⁺ growth and the ability to turn blue with a filter assay. Secondary positives were identified for further analysis. Proteins encoded by positive cDNAs were designated as HIPs (Huntingtin Interactive Proteins). Approximately 4.0 x 10⁶ Trp/Leu auxotrophic transformants were screened and of 14 clones isolated 12 represented the same cDNA (HIP 1), and the other 2 cDNAs, HIP2 and HIP3 were each represented only once. --

At page 13, lines 4-16, please amend as follows:

-- Liquid P-galactosidase assays were perfon-ned by inoculating a single yeast colony into appropriate synthetic complete (SQ dropout media and grown to OD600 0.6-1.5. Five millilitres of overnight culture was pelleted and washed once with I in) of Z-Buffer, then resuspended in 100 ml Z-Buffer supplemented with 38 mM 2-mercaptoethanol, and 0.05% SDS. Acid washed glass beads (-100 ml) were added to each sample and vortexed for four minutes, by repeatedly alternating a 30 seconds vortex, with 30 seconds on lee. Each sample was pelleted and 10 ml of lysate was added to 500 ml of lysis buffer. The samples were incubated in a 30-°C waterbath for 30 seconds and then 100 ml of a 4 mg/ml o-nitrophenyl b-D galactopyrano side (ONPG) solution was added to each tube. The reaction was allowed to continue for 20 minutes at 30-°C and stopped by the addition of 500 ml of I M Na₂CO₃ and placing the

samples on lee. Subsequently, OD420 was taken in order to calculate the P- galactosidase activity with the equation $1000 \times \text{OD420} / (t \times V \times \text{OD600})$ where t is the elapsed time (minutes) and V is the amount of lysate used. --

At page 14, lines 4-15, please amend as follows:

-- Subsequently, primer walking was used to determine the remaining sequences. A human frontal cortex >4.0 kb cDNA library (a gift from S. Montal) was screened to isolate the full length HIP I gene. Fifty nanogram of a 558 base pair Eco RI fragment from the original HIP I cDNA was radioactively labeled with [³²P]-dCTP using nick- translation and the probe allowed to hybridized to filters containing >10⁵ pfu/ml of the cDNA library overnight at 65-°C in Church buffer (see Northern blot protocol). The filters were washed at 65-°C for 10 minutes with 1 X SSPE, 15 minutes at 65-°C with 1 X SSPE and 0.1% SDS, then for thirty minutes and fifteen minutes with 1 X SSPE and 0.1 % SDS. The filters were exposed to X-ray film (Kodak, XAR5) overnight at -70-°C. Primary positives were isolated and replated and subsequent secondary positives were hybridized and washed as for the primary screen. The resulting positive phage were converted into plasmid DNA by conventional methods (Stratagene) and the cDNA isolated and sequenced. --

At page 15, lines 26-30 and Page 16, lines 1-3, please amend as follows:

-- Hybridization of the Northern blot with b-actin as an internal control probe provided confirmation that the RNA was intact and had transferred. The 1.2 kb HIP I cDNA was labeled using nick translation and incorporation of ³²P-dCTP. Hybridization of the original 1.2 kb HIP I cDNA was carried out in Church buffer (0.5 M sodium phosphate buffer, pH 7.2, 2.7% sodium dodecyl sulphate, 1 mM EDTA) at 55-°C overnight. Following hybridization, Northern blots were washed once for 10 minutes in 2.0 X SSPE, 0.1% SDS at room temperature and twice for 10 minutes in 0.15 X SSPE, 0.1% SDS. Autoradiography was carried out from one to three days using Hyperfilm (Amersham) film at -70-°C.

Page 17, lines 17-30 and Page 18, lines 1-2, please amend as follows:

-- Frozen human tissues were homogenized using a Polytron in a buffer containing 0.25M sucrose, 20mM Tris-HCl (pH 7.5), 1 mM EGTA, 2mM EDTA supplemented with 10ug/ml of leupeptin, soybean trypsin inhibitor and 1mM PMSF, then centrifuged at 4,000rpm for 10' at 4-°C to remove cellular debris. 100-150ug/lane of protein was separated on 8%

SDS-PAGE mini-gels and then transferred to PVDF membranes. Huntingtin and HIP I were electroblotted overnight in Towbin's transfer buffer (25 mM Tris- HCl, 0.192M glycine, pH8.3, 10% methanol) at 30V onto PVDF membranes (Immobilon-P, Millipore) as described (Towbin et al, Proc. Nat'l Acad. Sci. (USA) 76: 4350-4354 (1979)). Membranes were blocked for 1 hour at room temperature in 5 % skim milk/ TBS (10mM Tris-HCl, 0.15M NaCl, pH7.5). Antibodies against huntingtin (pAb BKPI, 1:500), actin (mAb A- 4700, Sigma, 1:500) or HIP I (pAb HIP-pep 1, 1:200) were added to blocking solution for 1 hour at room temperature. After 3 x 10 minutes washes in TBS-T (0.05% Tween-20/TBS), secondary Ab (horseradish peroxidase conjugated IgG, Biorad) was applied in blocking solution for 1 hour at room temperature. Membranes were washed and then incubated in chemiluminescent ECL solution and visualized using Hyperfilm-ECL film (Amersham).